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TITLE: Mammary Specific Expression of Cre Recombinase Under the Control of an Endogenous MMTV LTR: A Conditional Knock-out System

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13. ABSTRACT *(Maximum 200 Words)*

This project was designed to test expression of cre recombinase directed by the endogenous Mtv-17 locus. The hypothesis of the project was to develop a novel and useful breast cancer model using the tissue-specific expression of the Mtv-17 locus, which had previously been shown by our lab to express only in the mammary gland. We proposed targeting the cre recombinase gene to the Mtv-17 envelope gene, so that this enzyme would only be expressed in mammary epithelial cells. During the funding period, we were able to create ES cells that had the cre recombinase gene targeted to the Mtv-17 locus. However, we were unable to generate transgenic mice that retained this targeted gene in their germline. We were therefore unable to carry out the rest of the project, which was to cross the cre-recombinase-containing mice with mice that had the p53 gene flanked by the loxP target sequences, thereby creating mammary gland-specific loss of the p53 gene. We were thus unable to test our mammary tumor model.

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Introduction

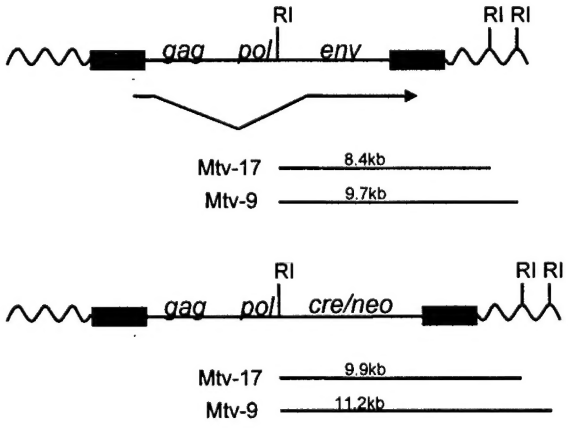
The inability to target expression of certain oncogenes only to the mammary gland has complicated their study in transgenic mouse models. This is especially important with regard to the role of certain tumor suppressors, such as p53, where mice with germline, targeted mutations generally die from other tumor types before they develop breast cancer. To overcome these problems, which as at the beginning of this project still remains a complication of many studies, we designed a knock-in vector that would direct expression of transgenes only to the mammary gland. This would allow for genetic manipulations in vivo that would not affect embryonic development or the function of other tissues. These vectors take advantage of an observation made in our lab that one of the endogenous mouse mammary tumor virus loci, Mtv-17, is expressed only in mammary gland and not other tissues of both virgin and lactating mice. We planned to knock-in the cre recombinase gene into the Mtv-17 locus, resulting in cre enzyme activity only in the mammary gland. The plan was then to cross these mice with mice engineered to have the cre recombinase target lox P sites flanking the p53 tumor suppressor gene. The resulting offspring of these matings would have been mice with targeted deletion of p53 only in mammary tissue. The prediction is that these animals would only develop mammary and not other tumors. We believed that the cre-transgenic mice would also prove useful for other investigators in the mammary gland development and tumorigenesis field.

Body

Targeting the Cre recombinase gene to the Mtv-17 locus

A vector was constructed which placed the cre recombinase gene in the envelope gene of *Mtv-17*. This construct was electroporated into C57Bl/6 embryonic stem cells and these were selected for neomycin resistance. The clones were first screened by polymerase chain reaction using one primer in virus flanking sequences not present in the targeting clone and the other primer in the neomycin resistance gene (Fig. 1). Four clones were positive by this assay. These clones were then expanded and DNA was isolated from each of them. This DNA was digested with EcoRI and probed with either probe specific for MMTV or for the Cre recombinase gene. One of the four clones, #114, had the correct band of about 10kb, that hybridizes both with the MMTV and cre probes (Fig. 2). This clone also has an additional band of about 11kb.

Fig. 1



During the past year, tried using clone #114 to create chimeras. Unfortunately, although the ES cells bearing this construct looked undifferentiated in culture, they did not result in chimeric mice after injection into blastocytsts (approximately 50 mice have been born when the 114 ES cell was used to generate chimeras with C3H/HeN blastocysts and none were chimeric for coat color). Thus, because we were unable to create mice with targeted expression of cre recombinase, we could not continue the original proposed studies. If we continue this project, we will have to re-introduce the targeting vector into new ES cells and rescreen such cells for targeted integration.

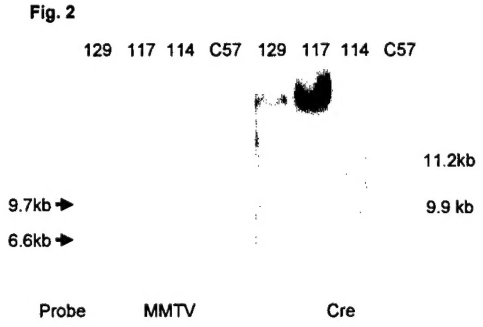


Fig. 2. Southern blot of ES cell clones electroporated with targeting vector 1. EcoRI-digested DNA from three different clones (129, 117, 114), as well as C57BL/6 DNA is shown. The endogenous MMTV bands of 9.7kb and 6.6kb are marked by black arrows; the targeted bands of 11.2 and 9.9 are marked by white arrows.

A second vector was also created to target cre recombinase to the Mtv-17 locus. In this vector, cre recombinase was placed in the gag gene of the endogenous provirus (Fig. 3). This vector was also transfected into C57BL/6 ES cells. However, after screening over 100 clones, we were unable to detect a homologous knock-in into the Mtv-17 locus.

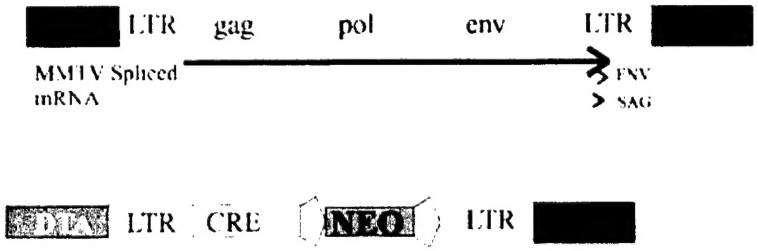


Fig. 3. Vector constructed to target cre receombinase to the gag gene of Mtv-17. The black boxes represent the host flanking sequences. Abbreviations: LTR, long terminal repeats; CRE, cre recombinase; NEO, neomycin resistance gene; DTA, diptheria toxin A chain gene.

The final vector that we constructed was designed to target the p53 locus (Fig. 4). This vector was designed to create a p53 gene with exons 5 and 6 flanked by lox P sites that are targets for the cre recombinase enzyme. Thus, mice bearing this construct would have normal p53 expression. However, after mating to a mouse expressing a tissue-specific cre recombinase, the p53 gene would be deleted in those tissues. This construct was completed but has not yet been introduced into ES cells.

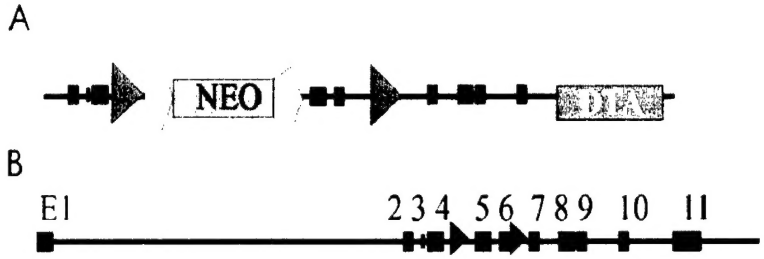


Fig. 4. The p53 knock-in vector (A) and endogenous p53 locus following homologus recombination of the vector (B). The lox p sites (red arrows) are embedded in the introns (represented by solid lines) and the exons are represented by solid boxes. Abbreviations: see Figs. 1 and 3.

Key Research Accomplishments

1. Creation of the two constructs needed to produce mice with mammary gland specific expression of p53.

2. Creation of the p53 knock-in vector.

Reportable Outcomes

Dr. Reb Russell III, originally supported by this grant, obtained a job in industry.

Dr. Jennifer Czarneski, supported in the second year of funding, obtained an NRSA postdoctoral fellowship from the National Institutes of Health.

Conclusions

While the approach to creating mammary gland specific expression of the cre recombinase remains feasible and worthwhile, there were a number of technical problems that precluded the final testing of the model. These problems were exacerbated by the change in personnel. With each new individual that joined the project, there was a period of "down-time" while that person retooled.

References

None

Appendices

None